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Ion uptake modified plants and DNA sequences for coding same

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ABSTRACT

There is provided a method of modifying an ion uptake characteristic of plant including the steps of identifying and isolating a gene responsible for an ion uptake mechanism, transforming a plant cell with a genetic construct including said gene, and culturing said transformant to produce a plant. The isolated gene is a K^+ channel or transporter. Sodium competes with potassium in transport or uptake, and in high sodium environments the amount of potassium entering the plant may be significantly impaired. Over expression of a K^+ transporter may permit survival or normal growth in high sodium environments such as marginal, high salinity soils. Specific mutation of a K^+ channel may permit selected uptake of potassium without the inhibition by sodium ions.

ION UPTAKE MODIFIED PLANTS AND DNA SEQUENCES CODING FOR SAME

This invention relates to ion uptake modified plants and DNA sequences coding for same.

This invention has particular but not exclusive applications to an ion uptake modified plants and DNA sequences coding for same, such as potassium channel and transporter genes whose introduction to the plant genome modifies the degree of uptake of potassium, and for illustrative purposes reference will be made to such application. However, it is to be understood that this invention could be used in other applications, such as modifying plants to alter other osmotically driven movements, pH tolerance, cation nutrition, loading into the xylem or cytosolic volume control.

Abiotic stresses such as drought, cold, salinity and potassium deficiency can have harmful effects on plants. For example, large areas of land are affected by high salinity, limiting land use for agriculture and commercial forestry. In Australia particularly, the growing of timber and pulp species, including *Eucalyptus* species and pines, is limited by the lack of salt tolerance of such species. Not being bound by theory, it is understood that in high salt environments there is an osmotic imbalance in plants, which leads to reduced plant growth or death. Further to osmotic imbalance, there is a reduction in essential macronutrients such as potassium. Sodium competes with potassium in transport or uptake, and in high sodium environments the amount of potassium entering the plant may be significantly impaired. Over expression of this potassium transporter may permit survival or normal growth in high sodium environments such as marginal, high salinity soils.

The present invention aims to alleviate at least one the above disadvantages and to provide ion uptake modified plants and DNA sequences coding for same which will be reliable and efficient in use. Other objects and advantages of this invention will hereinafter become apparent.

In one aspect, this invention resides broadly in a method of modifying an ion uptake characteristic of a plant and including the steps of:

identifying and isolating a gene responsible for an ion uptake mechanism;

transforming a plant cell with a genetic construct including said gene; and

culturing said transformant to produce a plant.

The ion uptake characteristic may be modified to improve the plant's tolerance to abiotic stresses such as drought, low K^+ concentration, salinity, cold and the like. Modifying an ion uptake characteristic of a plant may improve growth.

Suitably, the gene responsible for an ion uptake mechanism is responsible for a potassium (K^+) channel or transporter. A plant in accordance with the invention may be salt tolerant or may have improved growth in potassium deficient soils. By "salt tolerant" it is meant that a specified plant is capable of withstanding a higher level of salinity stress than the corresponding wild-type plant under typical growth parameters. Under the term K^+ channel or transporter it is be understood to include a corresponding DNA or cDNA sequence of the gene responsible for the K^+ channel or transporter.

Suitably, the K^+ channel is an inward-rectifying K^+ channel. The K^+ channel may be identified and isolated using any suitable standard molecular biology techniques common to the art. The plant may be exposed to selected environmental conditions to induce a desired gene/s. For example, the plant may be treated with a relatively high level of NaCl to possibly induce genes that may be involved in ion transport and the salt tolerant phenotype. The RNA may be isolated and used to construct a cDNA library. The library may be screened with suitable probes to identify desired target gene/s, such as primers from consensus sequences of known potassium channels.

The cDNA of the isolated potassium channel may be introduced into a genetic construct capable of expressing the introduced DNA and being transformed, such as a transformation vector appropriate for its application. It is to be appreciated that a person skilled in the art would be able to select a suitable vector and introduce the DNA sequence of interest using protocols common to the art.

In order to initiate the expression of the gene of interest, regulatory elements such as promoters may be included in the genetic construct. The regulatory element may include a constitutive promoter, such as the 35S-CaMV promoter, to enable expression of the gene in all cell types. The regulatory element may also be an inducible promoter, such as the endogenous promoter, that may be inducible in response to specific environmental stimuli. Alternatively, the regulatory element may be a tissue specific promoter or a promoter that may be expressed during specific developmental periods.

The plant may be transformed using methods common to the art, including but not limited to biolistics and *Agrobacterium* infection mediated gene transfer. Stable transformed plants may be selected using standard selection methods, including but not limited to the inclusion of antibiotic resistant genes for selection of transformants in antibiotic containing media. Stable transformed plants can be propagated using methods common to the art including micropropagation and breeding using judicious selection of parents. These parents may be untransformed, transformed with the same genetic construct, mutant variations of the gene of interest or distinct genes.

Preferably, the K^+ channel is isolated from *Eucalyptus camaldulensis* roots.

Accordingly, in a further aspect this invention resides in the isolated DNA sequence of the EcKT1 as set forth in SEQ ID No. 1. It is to be understood that the name EcKT1 and EKT1 are the same.

1. Sequence (Seq. ID No. 1):

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R = A or G
Y = C or T
S = G or C
K = G or T
H = A, C or T
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GGTGAGAATG AAATGTCAAC GAGCTCAACT GTTGAGCACA AGAAAAAGAA
AACAGGGGAA TTCTTCTSTG TCTGAAAACT GTTGTGTTGT GCCGTGAAGA
AGAAGAAGAA GCAGAGCATG GAGGGTTTGA TGCGAAACAG GGGAGGGGTG
CTGTGCGGAG TGTCGCTCTC GGTGTGCGGT CAGGAGGAGA TGCAGGCCTT
CTCCAGAGAC GGGAGCAGCC AATACAGCCY CGCCACCGGC ATCTTGCTGT
CCCTCGGCGC GAGGAGCAAC CGCGYCGTCC AGCTCCGCAG CTTCATCGTG
TCCTCCCTCG ATCGTCGTTA CAGGATATGG GAGAATTTCC TGGTTCTTCT
GGTTGTATAC ACTGCTTGGG CGTCGCCRTT CGAGTTCGGC TTCCTCAAGA
AGCCGAAGCC GCCGCTGTCC ATCATTGACA ATGTCGTCAA TGGATTCTTT
GCTATCGATA TCGTCCTTAC CTTCTTCGTG GCTTACCTCG ACAAAGCCAC
CTACCTACTT GTCGACGACC CGAAAAAGAT TGCTTGGAGG TACAYGACCT
CATGGTTCGC CCTTGATGTC ATATCGAYCA TTCCCTCCGA ACTTGCTCAG
AAAATCTCGC ATTCGCCTCT CGGGGGCTAT GGCCTATTCA ACATGCTCCG
ACTTTGGCGT CTCCGWAGGG TCAGCGCCCT GTTTTCGAGA TTGGAGAAAG
ATAGGAACTA TAACTACTTT GTGCTCCGGT GTGCAAAACT CCTCTGTGTT
ACTGTGTTTG CAGTCCATTG TGCAGGATGC TTCTATTATC TTCTCGCYGC
TCGTAATCAT GACCCGGCAG AAACTTGGAT GGGGAAAGCC ATTCTCCACG
ATGGCTTGGG GATCCGGTAT GTGACTTCGC TTTACTGGTC TATTACTACT
TTAACAACGG TTGGATATGG CGATCTGCAC CCAGTGAATG TGAGGGAGAT
GCTTTTCGAC ATATTCTTCA TGCTTTTCAA CCTCGGTTTG ACGGCATACT
TGATTGGTAA TATGACGAAC TTGGTTGTTC ACGGTACGAG TCGCACCAGA
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AAATTTAGGG ATACAATACA AGCTGCCTCA AGTTTTGCTC AAAGGAACCA
GTTGCCTCCT CGGCTACAAG ATCAGATGCT TGCTCATTTG TGCCTCAAGT
ATAGGACAGA TTCTGAGGGA CTTCGGCAAA AAGAGACTCT CGATTCTCTC
CCTAAAGCCA TCCGATCCAG CATCTTACAT TATCTTTTTT ACAATATCGT
CGACCRGGTG TACTTATTCC GTGGCATTTC AAATGACTTG CTCTTTCAGC
TGGTCTCAGA AATGAAAGAC GAGTATTTTC CTCCAAATGA AGATGTGATC
TTGCAAAATG AAGCACCAAC AGACTTCTAC ATTCTCGTCA CTGGTGCTGT
GGATCTGCTA GTTGTTAAAA ATGGAACTGA ACAGCCTGTT GGAGAGGCAA
AAAGTGGTGA TATTTGCGGG GAGATTGGTG TACTTTGCTA CAGGCCACAG
CTTTTCACAG TGCGGACGAA ACGATTGAGC CAGCTGCTAC GGCTAAATCG
TACCACATTG TTCAATATAA TCCAGTCAAA TGTTGAAGAT GGGACCATTA
TCATGAACAA TCTCCTTCAG CACTTGAAGG ACCTCAAGGA TCCAACCATG
GAGAGCATTC TGATTGACGC AGAGAACATG GTGGCACACG GTCAATTGGA
CCTGCCTCTC AGTTTATGCT TTGCCACATT GAGAGGAGAC RACTTGATGT
TGAGCCAACC ACTGAGACGG GGCCTAGATC CTAATGAATC AGATAACAGT
GGGAGGACAC CTCTGCATAT CGCAGCATCT AAAGGAAGCG AGAATTGCGC
GCTCCTCCTT ATGGATTATG GTGCAGATCC TAATAGCCGA GATTCAGAAG
GAAATGTGCC TCTTTGGGAG GCAATAAAGG GCGGTAGTGA ACCCGTGGTC
AAGTTGCTAG CASAAAATGG TGCTAATTTA GTATCTGGAG ACGTGGGTCA
ATTTTCATGC ACGGCAGCAG AACAGAATAA TCTGGACTTG CTCAAGGAGA
TCAGTCGTTA TGGTGGGGAT GTTACGCTTC CAAAAAGCAA CGGGACCACC
GCTCTTCATG TCGCTGTTTC TGARGATAAC ATTGAGATAG TCAAGTTCCT
CTTGGATAGA GGGGCAGACA TCAACAAACC GGACATCCAT GGATGGACAC
CCAGAGATTT AGCAGATCAA CAGGGACACG AAGAGATAGG AATTCTTTTT
CAATCCATAA ARGAAACTGA AATGCCKCCT GCTATTAGAA GCGAACCCAA
TTTGCCTCCT GCATCCCAAG AAAAAACTGT TGATGCTGCT TTTGGCCAAA
GCCGTCCAAG GAGGAGGACG AGTAACTTCC ACAATTCCTT GTTTGGCATT
ATGTCAGCTG CACGTGATGG TGAGGGAGAT GTGCTTTTAA GCATTAATCA
TAATAAATCT GCCAATAAGC CTTTTGTGGC ACGACCGGCT ATCCGTGTGG
TTGTTAGTTG TCCTGAAGTG GAGATGTTG AGGGGAAGCT TATGCTACTC
CCCGAGAACT TTCAGGAGTT GTTGGAGATG GCACGTAAGA AATTTGGGCT
TACTCTGCTC AAAGTTTTAA CCAAAAATGG AGCTGAAATT GATGATGTAG
CAGTTATCAG GGATGGCGAC CATCTAGTCT TTTTGAGCAA GGAAGTGAAG
GATGATTTTG CCAACAGAAA TGACAAGAAG GTCCAATAAG GAAGTGCATA
GGATTTTTCC TTCCGAATCA GTTTCCATGT CCACATCTTA GTCTAATGGA
AAGTCTAGTA CGACTGTTTC CATTATCTTC TTTATGTTCT TTCGCTCTTT
TTACATGAAT CAACACAAGG AACTGTACAT GTACTATGTA AGTAATCTGA
AGAAAATTCA YGTTATTCC
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The predicted expressed amino acid sequences for D and E isoforms of the EcKT1 gene are respectively listed as set forth in Seq. ID Nos. 2 and 3.

2. Sequence (Seq. ID No. 2):

```
MEGLMRNRGG VLCGVSLSVC GQEEMQAFSR DGSSQYSLAT GILLSLGARS
NRVVQLRSFI VSSLDRRYRI WENFLVLLVV YTAWASPFEF GFLKKPKPPL
SIIDNVVNGF FAIDIVLTFF VAYLDKATYL LVDDPKKIAW RYMTSWFALD
VISIIPSELA QKISHSPLGG YGLFNMLRLW RLRRVSALFS RLEKDRNYNY
FVLRCAKLLC VTVFAVHCAG CFYYLLAARN HDPAETWMGK AILHDGLGIR
YVTSLYWSIT TLTTVGYGDL HPVNVREMLF DIFFMLFNLG LTAYLIGNMT
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NLVVHGTSRT RKFRDTIQAA SSFAQRNQLP PRLQDQMLAH LCLKYRTDSE
GLRQKETLDS LPKAIRSSIL HYLFYNIVDQ VYLFRGISND LLFQLVSEMK
DEYFPPNEDV ILQNEAPTDF YILVTGAVDL LVVKNGTEQP VGEAKSGDIC
GEIGVLCYRP QLFTVRTKRL SQLLRLNRTT LFNIIQSNVE DGTIIMNNLL
QHLKDLKDPT MESILIDAEN MVAHGQLDLP LSLCFATLRG DDLMLSQPLR
RGLDPNESDN SGRTPLHIAA SKGSENCALL LMDYGADPNS RDSEGNVPLW
EAIKGGSEPV VKLLAENGAN LVSGDVGQFS CTAAEQNNLD LLKEISRYGG
DVTLPKSNGT TALHVAVSED NIEIVKFLLD RGADINKPDI HGWTPRDLAD
QQGHEEIGIL FQSIKETEMP PAIRSEPNLP PASQEKTVDA AFGQSRPRRR
TSNFHNSLFG IMSAARDGEG DVLLSINHNK SANKPFVARP AIRVVVSCPE
VGDVEGKLML LPENFQELLE MARKKFGLTL LKVLTKNGAE IDDVAVIRDG
DHLVFLSKEV KDDFANRNDK KVQ
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3. Sequence (Seq. ID No. 3):

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MEGLMRNRGG VLCGVSLSVC GQEEMQAFSR DGSSQYSPAT GILLSLGARS
NRAVQLRSFI VSSLDRRYRI WENFLVLLVV YTAWASPFEF GFLKKPKPPL
SIIDNVVNGF FAIDIVLTFF VAYLDKATYL LVDDPKKIAW RYTTSWFALD
VISTIPSELA QKISHSPLGG YGLFNMLRLW RLRRVSALFS RLEKDRNYNY
FVLRCAKLLC VTVFAVHCAG CFYYLLAARN HDPAETWMGK AILHDGLGIR
YVTSLYWSIT TLTTVGYGDL HPVNVREMLF DIFFMLFNLG LTAYLIGNMT
NLVVHGTSRT RKFRDTIQAA SSFAQRNQLP PRLQDQMLAH LCLKYRTDSE
GLRQKETLDS LPKAIRSSIL HYLFYNIVDR VYLFRGISND LLFQLVSEMK
DEYFPPNEDV ILQNEAPTDF YILVTGAVDL LVVKNGTEQP VGEAKSGDIC
GEIGVLCYRP QLFTVRTKRL SQLLRLNRTT LFNIIQSNVE DGTIIMNNLL
QHLKDLKDPT MESILIDAEN MVAHGQLDLP LSLCFATLRG DNLMLSQPLR
RGLDPNESDN SGRTPLHIAA SKGSENCALL LMDYGADPNS RDSEGNVPLW
EAIKGGSEPV VKLLALNGAN LVSGDVGQFS CTAAEQNNLD LLKEISRYGG
DVTLPKSNGT TALHVAVSED NIEIVKFLLD RGADINKPDI HGWTPRDLAD
QQGHEEIGIL FQSIKETEMP PAIRSEPNLP PASQEKTVDA AFGQSRPRRR
TSNFHNSLFG IMSAARDGEG DVLLSINHNK SANKPFVARP AIRVVVSCPE
VGDVEGKLML LPENFQELLE MARKKFGLTL LKVLTKNGAE IDDVAVIRDG
DHLVFLSKEV KDDFANRNDK KVQ
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Expression, or over expression of the EcKT1 gene may lead to improved nutrient uptake in marginal soils, and may also maintain a potassium balance in plants grown in high sodium chloride. Specific mutation of this channel may permit selected uptake of potassium without the inhibition by sodium ions. Accordingly, gene transfer of the EcKT1 genes into desired plants of interest may generate a salt tolerant plant or a plant which is able to grow in soils with low K^+ potassium content.

The isolated EcKT1 gene may be subcloned into the appropriate genetic vectors using standard molecular biological methods for expression in the desired target plant species. These cDNAs can be under the control of constitutive promoters, eg. 35S-CaMV, or tissue specific or inducible promoters including the endogenous promoter of said cDNAs. Thus, the isolation of new promoters has significant value to regulate the endogenous gene and/or other exotic genes of interest.

Accordingly, in a further aspect, this invention resides in the DNA sequence encoding the promoter of the EcKT1 gene as set forth in Seq. ID No. 4.

4. Sequence (Seq. ID No. 4):

```
M = A or C
R = A or G
W = A or T
S = C or G
Y = C or T
K = G or T
V = A, C or G
H = A, C or T
D = A, G or T
B = C, G or T
X or N = A, C, G or T

CGACGGCCCG GGCTGGTCTG GGCTTTTTCT GATTGTCCAT CCAAGTGCAC
GTGGCAGCTT TTGAACCTTB GCCATTAGTA TCGATGGTTG GGATTTGCTC
TTTCTTTGAT CTGATGGTCA AGAGACTGGG ATCCATTCCC GAAGAATAAG
ATGTTACTAG TCGAGATTCT CTTTTTCTCT CCGACAGTTA TCGAGGGATT
AGTGATCCAT TCCCCAAKAA TTGTTGTGTT AAGATATGTC TTTCTAGAGT
CATGTATAWT TAGCGCATTG RAAGTTAAAA TATTTTCTTA CCTAGTGTAC
TTGAAGAGTA CTCSAAACAC CYGTTWAATG AGACCTTTAT ATGTATAAGT
CATGTTTAAT GCRCTTAACG ARACTTTGCC GCATCATATC ACAAATGGAT
CATACAAATA AATTTGAGRG TATGTTTGAA ATTATCATAA TCATGTCTTG
AAAAAATATT AACACATGTA TATGAATAAT AATAAATAAT AAAAAAACAG
CGATGTTTYC CRTWCTCCTA GGSTGGAAGA AACATTTTCC AACCATGGCA
CCGATTGATA TGTTGTCATT GGTRATTAAA TTCACAAAAA TGGCGGTGCT
TATATTTGTA ATCATTARTG GTGGAGAAAA AACACAACCC TAATTGCAAT
GATATAATTG TAGCRCGCGG AATACTTCAA ACATCTTATA AAAACTACAA
AATTAATTTC TTTTTTCTAT GTAGTGCATT GAAAAACTGT GCCGAAGATT
TTCCGATAGT TCATCAGCAC AACTTCTTAC GTSGGTTTCA ACTTTGAAAA
GGGCTAGTAC AGTACTTTCR TAGTGTTCAA CTTGGCAAGC ATCACAACTT
CTAGCYTCTK TTCGGAACCT GCCCCCACCC ACTTTTCAAT GATGCGACTT
CTAAATATAM ATAGCCAAAT ACAGTATACA CAACTGCGCA TGACTATACA
ATTTCAATTT TTTTTTCCAC CTGCCACTCT AGCACTTTTA GCACTTTGCA
ATAGAGTCAC ATCAAAATGG ATTCTTAGCG TCTCTAAAGT GATTATTCTT
TTTGGYAGTG ACTTTGATTT TRRATCCAYA TCTGATGACC TCAGGAGGAT
GCTGGACCAA TCCTGTCTTA TTTTTTATAT CTTCCGATTG CCGAAATGAC
GGGTCAYGGT CGGCAGGAGT GATGCACCCA TGGGAAGTAC ACTGTGCAAC
ACAGGAGAGA KAGAKAGAGA GAGATTCTTG ATATTCTTGA TGTGAGAATG
AAATGTCAAC GAGCTCAACT GTTGAGCACA AGAAAAAGAA AACAGGGGAA
TTCTTCTSTG TCTGAAAACT GTTGTGTTGT GCCGTGAAGA AGAAGAAGAA
GCAGAGCATG
```

The DNA sequence of the EcKT1 promoter as set forth in SEQ ID No. 4 contains ambiguity codes in addition to the four bases (A, G, C, T), as there may be more than one sequence. A single DNA sequence of the EcKT1 may be isolated from tissues of plants which have been transformed with the EcKT1 promoter.

Accordingly, in a further aspect, this invention resides in the DNA sequence encoding the promoter of the EcKT1 gene as set forth in Seq. ID No. 5.

5. Sequence (Seq. ID No. 5):

```
S = G or C
The last 3 bases (ATG) represent the protein translation
initiation codon.
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```
CGACGGCCCG GGCTGGTCTG GGCTTTTTCT GATTGTCCAT CCAAGTGCAC
GTGGCAGCTT TTGAACCTTG GCCATTAGTA TCGATGGTTG GGATTTGCTC
TTTGTTTGAT CTGATGGTCA AGAGACTGGG ATCCATTCCC GAAGAATAAG
ATGTTACTAG TCGAGATTCT CTTTTTTCTT TCCGACAGTT ATCGAGGGAT
TAGTGATCCA TTCCCCAAGA ATTGTTGTGT TAAGATATGT CTTTCTAGAG
TCATGTATAA TTAGCGCATT GAAAGTTAAA ATATTTTCTT ACCTAGTGTA
CTTGAAGAGT ACTCGAAACA CCCGTTTAAT GAGACCTTTA TATGTATAAG
TCATGTTTAA TGCGCTTAAC GAGACTTTGC CGCATCATAT CACAAATGGA
TCATACAAAT AAATTTGAGG GTATGTTTGA AATTATTATA ACTATGTCTT
GAAAAAATAT TAACACATGT ATATGAATAA TAATAAATAA TAAAAAAACA
GCGATGTTTC CCGTACTCCT AGGCTGGAAG AAACATTTTC CAACCATGGC
ACCGATTGAT ATGTTGTCAT TGGTGATTAA ATTCACAAAA ATGGCGGTGC
TTATATTTGT AATCATTAGT GGTGGAGAAA AAACACAACC CTAATTGCAA
TGATATAATT GTAGCGCGCG GAATACTTCA AACATCTTAT AAAAACTACA
AAATTAATTT CTTTTTTCTA TGTAGTGCAT TGAAAAACTG TGCCGAAGAT
TTTCCGATAG TTCATCAGCA CAACTTCTTA CGTGGGTTTC AACTTTGAAA
AGGGCTAGTA CAGTACTTTC GTAGTGTTCA ACTTGGCAAG CATCACAACT
TCTAGCCTCT GTTCGGAACC TGCCCCCACC CACTTTTCAA TGATGCGACT
TCTAAATATA AATAGCCAAA TACAGTATAC ACAACTGCGC ATGACTATAC
AATTTCAATT TTTTTTTCCA CCTGCCACTC TAGCACTTTT AGCACTTTGC
AATAGAGTCA CATCAAAATG GATTCTTAGC GTCTCTAAAG TGATTATTCT
TTTTGGCAGT GACTTTGATT TTAAATCCAT ATCTGATGAC CTCAGGAGGA
TGCTGGACCA ATCCTGTCTT ATTTTATATC TTCCGATTGC CGAAATGACG
GGTCACGGTC GGCAGGAGTG ATGCACCCTG GGAAGTACAC TGTGCAACAC
AGTAGATAGA GAGAGATTCT TGATATTCTT GATGTGAGAA TGAAATGTCA
ACGAGCTCAA CTGTTGAGCA CAAGAAAAAG AAAACAGGGG AATTCTTCTS
TGTCTGAAAA CTGTTGTGTT GTGCCGTGAA GAAGAAGAAG AAGCAGAGCA
TG
```

In another aspect, this invention resides in a method of regulating expression of a gene including the steps of:

identifying and isolating a gene of interest;

constructing an expression cassette including said gene and a promoter selected from those promoting genes responsible for an ion uptake mechanism;

transforming a plant cell with said expression cassette;

culturing said transformant to produce a plant.

The expression cassette may be introduced into a plant of interest by transformation in order to initiate the expression of any suitable gene of interest

during conditions which result in a modified ion uptake mechanism in the plant. For example, these conditions may result in the expression of the gene of interest during low soil K^+ levels or high salinity. The promoter may be active in vascular and/or root tissues. The promoter may also be active in response to abscisic acid which is involved in plant adaptation processes to abiotic stresses.

The gene of interest may be responsible for expression of stress tolerance or insect resistance. Suitably, the promoter is the EcKT1 promoter.

In order that this invention may be more readily understood and put into practical effect, reference will now be made to the following figures and examples which illustrate preferred embodiments of the invention and wherein:

FIG. 1 is a phylogenic tree of plant K^+ channel proteins illustrating the relationship of the EcKT1 protein with other previously identified channel proteins from *Arabidopsis*, potato and maize. The phylogenic tree was produced with the Genetics Computer Group (GCC Inc., Madison, USA) programmes GrowTree, Distances and PileUp using neighbour-joining and Kimura Protein Distant algorithms;

FIG. 2 depicts a schematic representation of a possible association of EcKT1 with the extracellular membrane;

FIG. 3 depicts the EcKT1 expression in *E. camaldulensis* using Southern blot analysis;

FIG. 4 is a graph of the EKT1 transcript levels in Eucalypts,

FIG. 5 illustrates the GUS expression, representing an active EcKT1 promoter, in vascular tissue of transgenic *Arabidopsis* plants, and.

FIG. 6 depicts the GUS activity in root and aerial tissues of pEcKT1-GUS transgenic *Arabidopsis* plants.

<u>**EXAMPLE 1**</u>

The EcKT1 cDNA and promoter were isolated from *Eucalyptus camaldulensis* roots, which encodes a potassium channel and regulatory element respectively, using standard molecular biology techniques common to the art. *E. camaldulensis* plants were treated with 75 mM NaCl to possibly induce genes which are involved in salt tolerance. RNA was isolated and a cDNA library constructed. The EcKT1 gene was identified and isolated from this library by screening with a

radioactive probe generated from a PCR template using primers from a consensus sequence of known potassium channels.

EcKT1 is a low-affinity inward-rectifying K+ channel. The EcKT1 gene most closely resembles the *Arabidopsis* AKT1 gene in sequence databases as illustrated in FIG. 1. A schematic representation of possible association of EcKT1 with the extracellular membrane is illustrated in FIG. 2. It is to be appreciated that this diagram is an example and is not intended to represent the only possible membrane association configuration of the EcKT1 protein. The DNA sequence of two EcKT1 isoforms and the predicted protein sequences are illustrated in Seq. ID No. 1 to 3. The cDNA represents the full length sequences of about 3.0 kilobase pairs of DNA, which encode a complete and functional protein.

The EcKT1 cDNA was cloned into a yeast expression vector (pYES2) and transformed into the K+ uptake-deficient (trk1, trk 2) *S. cerevisiae* strain CY162. The CY162 yeast cells were streaked on arginine-based medium supplemented with galactose, sucrose and 50 or 1 mM KCL and incubated for two days at 30^0C. *S. cerevisiae* strain DBY 746 was used as the wild type control. As shown in Table 1, the EcKT1 gene is able to complement the growth of a K^+ deficient yeast mutant allowing growth on standard yeast propagation media modified to contain a low concentration of K^+. This also confirms that the *E. camaldulensis* EcKT1 gene encodes a functional K^+ channel.

Table 1

	Wild Type	CY162 + pYES-EKT1	CY162 + pYES
50mM KCl	Growth	Growth	Growth
1mM KCl	Growth	Growth	No Growth

Southern blot analysis of the *E. camaldulensis* K^+ channel gene family was performed, as illustrated in FIG. 3. The total genomic DNA (2µg) isolated from *E. camaldulensis* tissue was digested separately with the DNA restriction enzymes BamH1, HinDIII or EcoR1. The fragments were separated by agarose gel electophoresis, blotted onto Qiabrane N^+ membrane and probed with an *E. globulus* K^+ channel PCR fragment (an EcKT1 radioactive probe) and exposed to X-ray film

for detection. Southern blot analysis using the low stringency conditions and an EcKT1 probe indicated a single gene in *E. camaldulensis*.

As illustrated in FIG. 4, EcKT1 expression levels in aerial and root tissue of *Eucalyptus* was assessed using RT-PCR. The level of EcKT1 transcript was normalised against α-tubulin levels under a limited number of PCR cycles. EcKT1 expression was induced in roots in response to Na^+ stress (75 mM) and K^+ starvation treatment alone and a combined treatment synergised the level of expression. There was no change in EcKT1 expression levels in aerial, non-root tissue with the Na^+ or K^+ treatments alone, and a slight reduction when treated in combination. These results suggest that the EcKT1 K^+ channel may play a role in K^+ uptake from the environment and is responsive to K+ deficient conditions.

EXAMPLE 2

The promoter, or regulatory element, of the EcKT1 gene was cloned. This regulatory element when subcloned 5' of the marker gene encoding β-glucuronidase (GUS) drives the expression of said marker gene in tissues associated with the plant vasculature. Such expression may be valuable when it is desired to express a gene in this specific region, such as genes encoding transport, channel or pore-like proteins in this region. For example, the promoter could target insect resistant genes to combat phloem feeding pests. The promoter would also be useful for specific targeting of stress tolerant genes.

The promoter of the EcKT1 gene was isolated using suitable molecular biology techniques that are common to the art. Nested PCR primers were used in a 'promoter finder' procedure to isolate the DNA sequences 5' of the coding sequence on genomic DNA clones. Three different DNA restriction enzymes were used to digest DNA that resulted in the cloning of three DNA fragments of sizes: 1.5 kilobases (Kb), PvuII; 0.8 Kb, ScaI; and 0.5 Kb, EcoRI. The 1.5 Kb and 0.5 Kb promoters were isolated from genomic clone 1.8 and the 0.8 Kb promoter from genomic clone 3. These fragments were cloned and sequenced as indicated in SEQ. ID No. 4.

The PCR fragments were cloned into the vector pBI101. The pBI101 plasmid is readily available and is designed for cloning and testing promoters in plants using β-glucouronidase (GUS) expression. The 0.8 Kb and 0.5 Kb fragments were

subcloned to drive the expression of the marker gene GUS. Positive GUS staining, representing active promoter DNA fragments, was visible in the vascular tissues of transgenic *Arabidopsis* plants, as illustrated in FIG. 5.

The transgenic plants in FIG 5 of the patent are stained for GUS marker gene expression. This gives an indication of which tissues the EcKT1 promoter is active in. Using the same transgenic *Arabidopsis* plants the level of marker gene expression was quantified using a standard fluorometric assay for GUS enzyme activity.

The DNA sequence of the EcKT1 promoter according to Seq. ID No. 4 contains ambiguity codes in addition to the four bases (A, G, C, T). This is because there was more than one sequence for the promoter. The DNA corresponding to the EcKT1 promoter from *Agrobacterium* cells containing the pEcKT1-GUS construct used to produce the transformed *Arabidopsis* plants was recovered and sequenced. The sequence of the promoter DNA recovered from Agrobacterium is given in SEQ ID No. 5 (5' to 3', top strand). There is still one ambiguity code (S = G or C). The last 3 bases (ATG) represent the protein translation initiation codon.

This stretch of 1352 nucleotide bases is responsible for controlling the expression pattern of the GUS (β-glucuronidase) marker gene in vascular tissues (Fig. 5). Having a sequence without ambiguity codes helps in identifying sequence motifs, known as cis-acting elements, which are potential binding sites for regulatory trans-acting factors.

Communication between the root and the shoot forms a central part of the coordinated response of plants to drought and salinity. Recent evidence suggests that an outward-rectifying K+ channel expressed in vascular tissue of the root may have a major role in this process (Gaymard et al., Cell 94, 647-655 1998; Hetherington Current Physiology 8 R911-R913 1998). The inward-rectifying EcKT1 K+ channel is also expressed in root vascular tissue and maybe involved in coordinating the plant's response to abiotic stresses such as drought and salinity.

The plant hormone abscisic acid (ABA) is involved in numerous physiological responses of the plant. These mediate adaptation processes to abiotic stresses, such as drought or water deficit, salt stress and in some cases mechanical stress. ABA-induced gene expression is an important part of ABA action. An analysis of the cis-acting sequences required for ABA-induced gene expression identified ABRE (ABA responsive element) sequences in promoters of many ABA-response

genes. The ABRE is similar to a family of sequences called the G-box, which also contain an ACGT core and are present in a number of gene promoters that respond to different environmental conditions. The EcKT1 promoter contains an ABRE (CACGTGGCA), which is underlined in Seq. ID No. 5.

The presence of an ABRE in the EcKT1 promoter suggests that the EcKT1 gene may be regulated in part by ABA and indicates that this gene may be involved in coordinating the response of plants to drought and salinity.

There are similarities in the expression pattern between the 1.352kb EcKT1 Eucalyptus promoter in *Arabidopsis* (FIG. 6) and the endogenous EcKT1 gene in *Eucalyptus* (FIG. 4).

- The expression level in aerial tissue does not alter much during salt stress or potassium starvation.
- The expression level in root tissue is significantly increased upon salt stress or potassium starvation.
- The 1.352Kb EcKT1 promoter conferred potassium starvation inducibility to the GUS marker gene.
- The 1.352Kb EcKT1 promoter conferred vascular tissue expression to the GUS marker gene.

It will of course be realised that while the above has been given by way of illustrative example of this invention, all such and other modifications and variations thereto as would be apparent to persons skilled in the art are deemed to fall within the broad scope and ambit of this invention as is herein set forth.

CLAIMS:

1. A method of modifying an ion uptake characteristic of a plant and including the steps of:

identifying and isolating a gene responsible for an ion uptake mechanism; transforming a plant cell with a genetic construct including said gene; and culturing said transformant to produce a plant.

2. A method according to claim 1, wherein the ion uptake characteristic is modified to improve the plant's tolerance to abiotic stresses selected from drought, low K^+ concentration, salinity, cold and the like.

3. A method according to claim 1, wherein the ion uptake characteristic is modified to improve the plant's growth.

4. A method according to any one of the preceding claims, wherein said gene is responsible for a K^+ channel or transporter.

5. A method according to Claim 4, wherein the K^+ channel is an inward-rectifying K^+ channel.

6. A method according to Claim 5, wherein the gene responsible for the K^+ channel is isolated from *Eucalyptus camaldulensis* roots.

7. A method according to Claim 6, wherein the gene is the EcKT1 gene.

8. A method according to Claim 7, wherein the isolated DNA sequence of EcKT1 is as set forth in SEQ ID No. 1.

9. A method according to anyone of the preceding claims, wherein the genetic construct includes a promoter selected from a constitutive, tissue specific or inducible promoter.

10. A method according to claim 9, wherein the constitutive promoter is 35S-CaMV promoter.

11. A method according to Claim 9, wherein said inducible promoter is the promoter of the EcKT1 gene.

12. A method according to Claim 11, wherein the DNA sequence of the promoter is as set forth in SEQ ID No. 4.

13. A method according to Claim 11, wherein the DNA sequence of the promoter is set forth in SEQ ID No. 5.

15. A method according to any one of the preceding claims, wherein said transformed plant is rendered salt tolerant.

16. A method according to anyone of the preceding claims, wherein said transformed plant is rendered able to grow in soils with low K^+ content.

17. The isolated DNA sequences of the EcKT1 gene as set forth in SEQ ID No. 1.

16. The DNA sequences encoding the promoter of the EcKT1 gene as set forth in SEQ. ID No. 4.

18. The DNA sequence encoding the promoter of the EcKT1 gene as set forth in SEQ. ID No. 5.

19. A method of regulating expression of a gene including the steps of:
identifying and isolating a gene of interest'
constructing an expression cassette including said gene and a promoter selected from those promoting genes responsible for an ion uptake mechanism;
transforming a plant cell with said expression cassette;
culturing said transformant to produce a plant.

20. A method according to Claim 19, wherein the gene of interest expresses in response to low soil K^+ ion levels.

21. A method according to Claim 19, wherein the gene of interest expresses in response to high levels of soil salinity.

22. A method according to any one of Claims 19 to 21, wherein the gene of interest expresses in vascular tissue.

23. A method according to any one of Claims 19 to 22, wherein the gene of interests expresses in the root.

24. A method according to Claim 23, wherein the gene of interest expresses in response to abscisic acid.

25. A method according to any one of Claim 19 to 24, wherein the gene of interest is responsible for expression of stress tolerance or insect resistance.

26. A method according to any one of Claims 19 to 25, wherein the promoter is EcKT1.

DATED THIS 27TH DAY OF SEPTEMBER 1999
FORBIO LIMITED
&
JACKSTOWN PTY LTD
BY
PIZZEYS PATENT & TRADE MARK ATTORNEYS

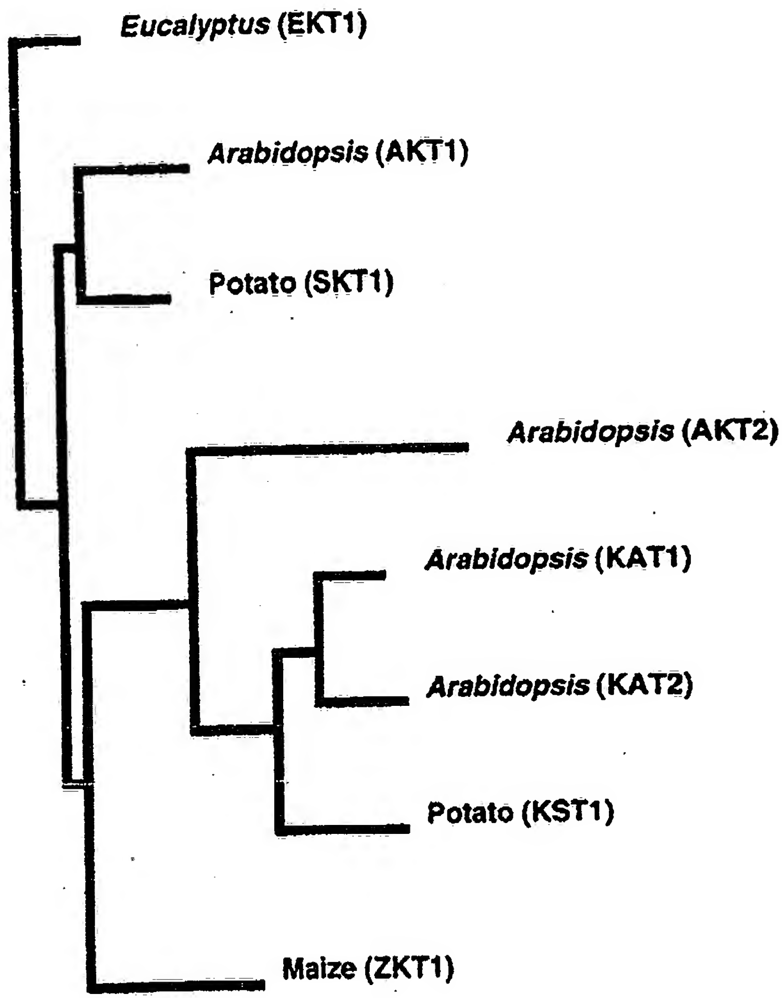
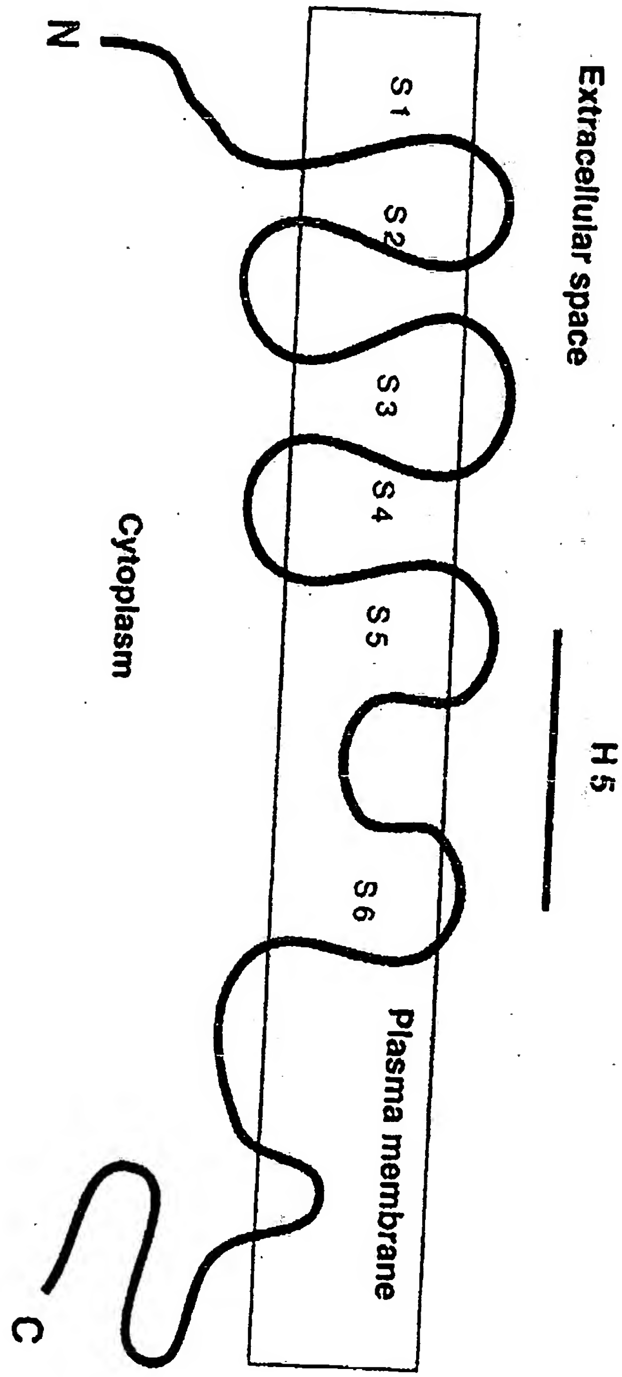

Eucalyptus (EKT1)
Arabidopsis (AKT1)
Potato (SKT1)
Arabidopsis (AKT2)
Arabidopsis (KAT1)
Arabidopsis (KAT2)
Potato (KST1)
Maize (ZKT1)

FIG. 1



27 09 95 50164

FIG. 2

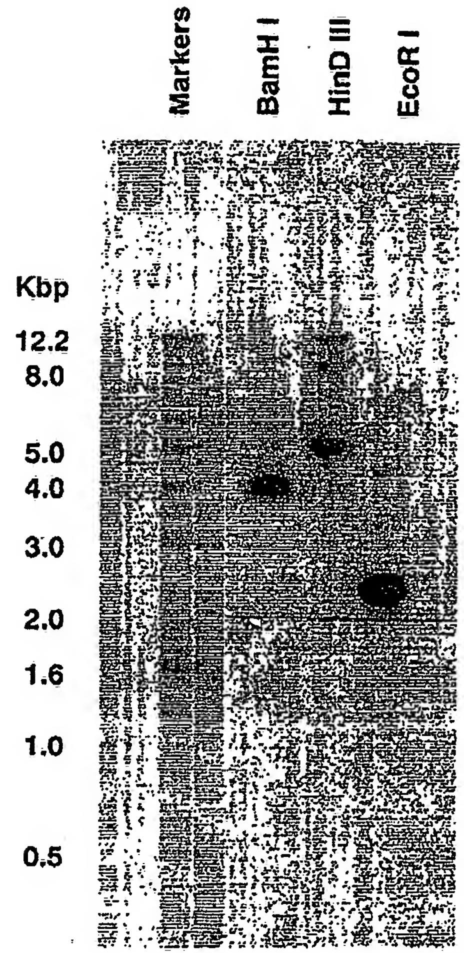


FIG. 3

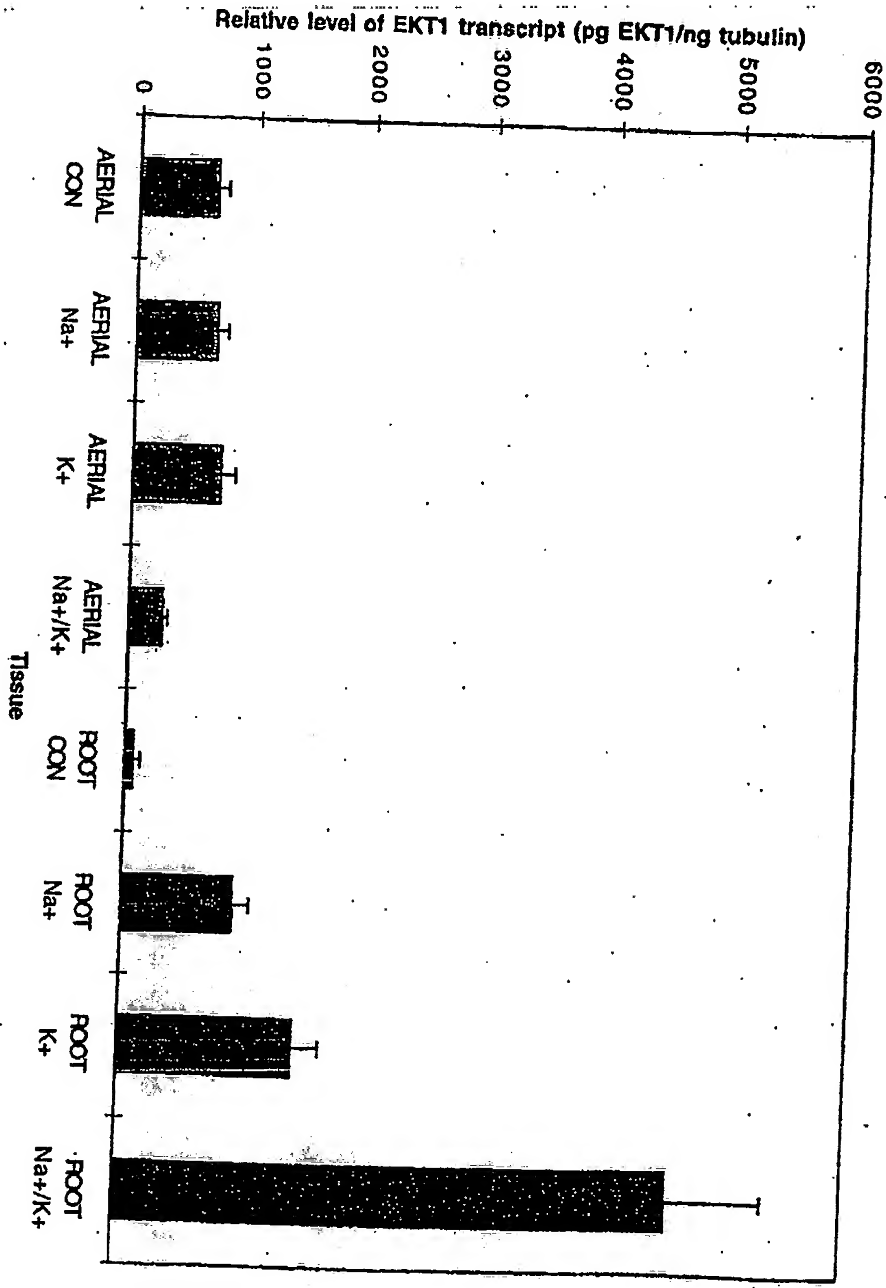

Relative level of EKT1 transcript (pg EKT1/ng tubulin)
0
1000
2000
3000
4000
5000
6000
AERIAL CON
AERIAL Na+
AERIAL K+
AERIAL Na+/K+
ROOT CON
ROOT Na+
ROOT K+
ROOT Na+/K+
Tissue

FIG. 4

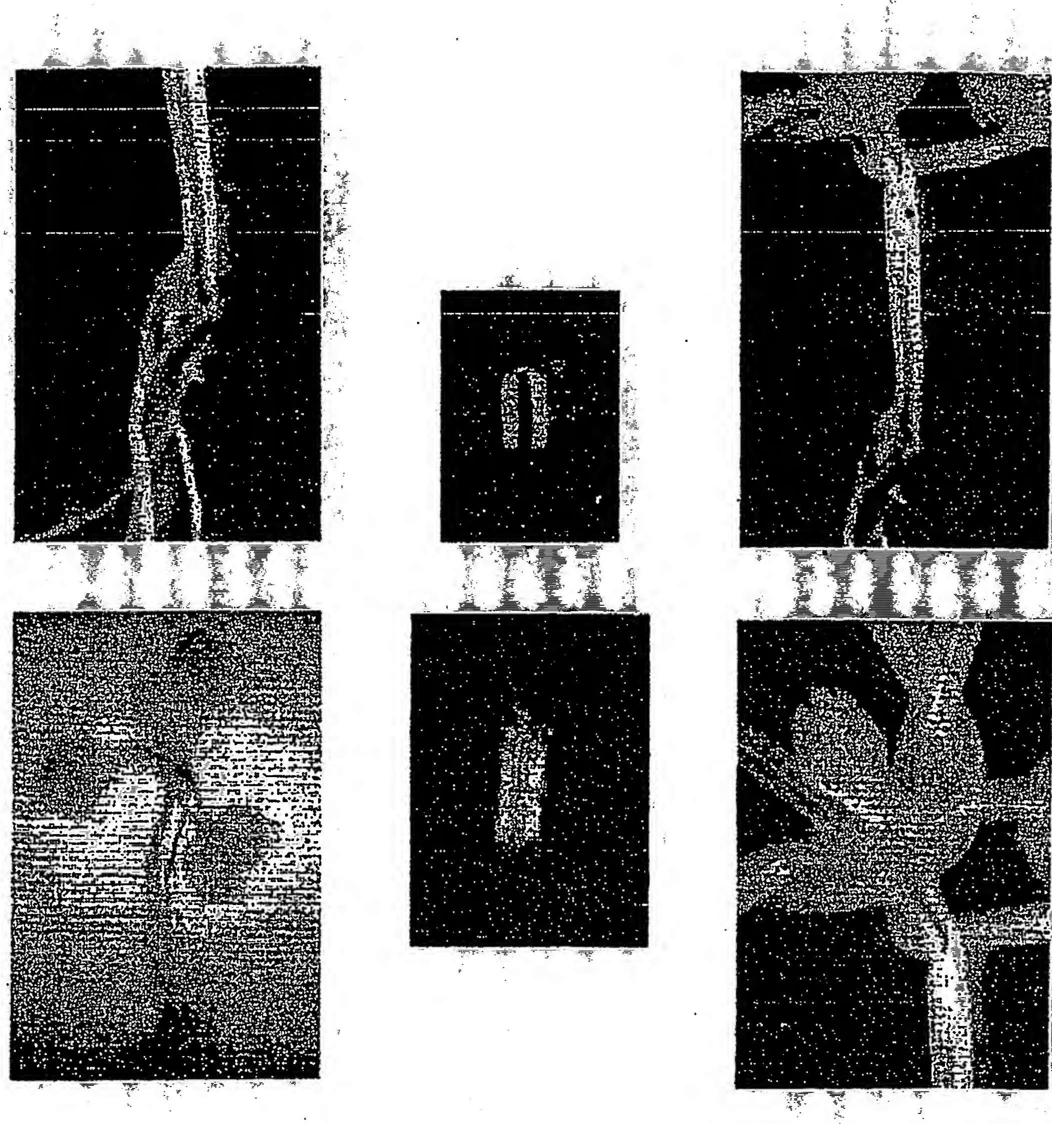
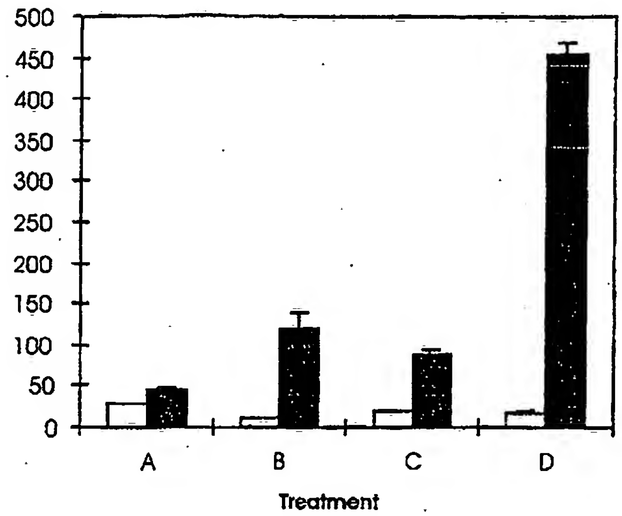


FIG. 5



Treatments A: Control.
B: Salt stressed (75 mM).
C: Potassium starvation and salt stressed (75 mM).
D: Potassium starvation.
Black bars: Root tissue.
Open bars: Aerial tissue.

FIG. 6